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Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) RsbU-1 derived from Staphylococcus

(57) isbU-l polypeptides and DNA (RNA) encoding such rsbU-1 and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such rsbU-1 forlm the treament of infection, particularly bacterial infections. Antagonists against such rsbU-1 and their use as a therapeutic to treat infections, particularly bacterial infections are also disclosed. Also disclosed are diagnostic assay for detecting diseases related to the presence of rsbU-1 nucleic acid sequences and the polypeptides in a host. Also disclosed are diagnostic assays for detecting polynucleotides encoding SigB operon and for detecting the polypeptide in a host.

EP 0 843 011 A2

Description

This application claims benefit to US Provisional application 60/029,118 filed 24 October 1996.

This invention relates, in part, to newly identified polynucleotides and polypeptides: variants and derivatives of these polynucleotides and polypeptides; processes for making these polynucleotides and these polypeptides, and their variants and derivatives: agonists and antagonists of the polypeptides; and uses of these polynucleotides, polypeptides, variants, derivatives, agonists and antagonists. In particular, in these and in other regards, the invention relates to polynucleotides and polypeptides of SigB operon, hereinafter referred to as "rsbU-1".

BACKGROUND OF THE INVENTION

Regulation of gene expression in bacteria occurs frequently at the level of transcription. RNA polymerases which transcribe these genes are composed of a multisubunit core enzyme and an additional protein, or sigma factor, which permits the whole enzyme to recognise promoter elements and initiate transcription at these specific sites. Cells contain multiple sigma factors and their relative levels in the cell provide a fundamental control of gene expression.

Bacillus subtilis has at least 10 different sigma factors [Haldenwang, 1995]. Sigma-B is activated to direct the transcription of a subset of genes when *B. subtilis* stops exponential growth or is subjected to a number of environmental stresses (eg heat, salt, ethanol and peroxide) [Boylan *et al,* 1993a,b].

S. aureus, is a pathogen related to *Bacillus* species. In the host *S. aureus* cells are exposed to a range of environmental stresses, some analogous to those mentioned above. Furthermore bacterial populations in infection loci are likely to contain slow or non-growing bacteria. Thus an equivalent of sigma-B is likely to play a crucial role in the adaptation of *.S. aureus* to the host environment.

At least four proteins are known to regulate sigma-B Three (RsbV, RsbW and RsbX) are the products of genes which are co-transcribed with the structural gene for sigma-B(sig*B*). The fourth (RsbU) lies immediately upstream of the *rsbV*gene. RsbW binds to sigma-B, blocking sigma-B-dependent transcription. RsbV can form a comples with RsbW and reduce the sequestration of sigma-B by RsbW. Additionall RsbW is able to phosphorylate RsbV to a form which is unable to interact with RsbW [Dufour & Haldenwang, 1995]. RsbU, directly or indirectly, facilitates the RsbV-dependent release of sigma-B from RsbW [Voelker *et al.*, 1995].

Kalman et al. report the similar organisation of the *sigB* and *spoIIA* operons encoding alternate sigma factors of *Bacillus subtilis*. J. Bacteriol. 172, 5575-85 (1980). Further, Wise & Price report that four additional genes in the sigB operon of *Bacillus subtilis* control actiivity of the general stress factor sigma-B in response to environmental signals. J Bacteriol. 177, 123-133 (1995).

Clearly, there is a need for factors that may be used to screen compounds for antibiotic activity and which may also be used to determine their roles in pathogenesis of infection, dysfunction and disease. There is a need, therefore, for identification and characterization of such factors which can play a role in preventing, ameliorating or correcting infections, dysfunctions or diseases.

The polypeptide of the present invention has sequence homology to known regulator of a sigma factor

Background References

Boylan, S.A., Redfield. A. R & Price, C.W. (1993a) Transcription factor sigma B of *Bacillus subtilis* controls a large stationary-phase regulon Journal of Bacteriology **175**, 3957-63

Boylan, S.A., Redfield. A.R., Brody, M.S & Price CW. (1993b), Stress-induced activation of the sigma B transcription factor of *Bacillus subtilis*, Journal of Bacteriology **175**, 7931-7

Dufour, A. & Haldenwang, W.G. (1995) Interactions between a *Bacillus subtilis* anti-sigma factor (RsbW) and its antagonist (RsbV). Journal of Bacteriology **176**, 1813-20.

Haldenwang, W.G. (1995). The sigma factors of *Bacillus subtilis*. ***Microbiological Reviews*** 59, 1-30.

Voelker, U., Dufour, A. & Haldenwang, W.G. (1995). The *Bacillus subtilis rsbU* gene product is necessary for RsbX-dependent regulation of sigma B. Journal of Bacteriology **177**, 114-22.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide polypeptides, *inter alia*, that have been identified as novel rsbU-1 by homology between the amino acid sequence set out in Figure 2 [SEQ ID NO: 2] and known amino acid sequences of other proteins such as Bacillus subtilis rsbU.

It is a further object of the invention, moreover, to provide polynucleotides that encode rsbU-1, particularly polynucleotides that encode the polypeptide herein designated rsbU-1.

In a particularly preferred embodiment of this aspect of the invention the polynucleotide comprises the region

encoding rsbU-1 in the sequence set out in Figure 1 [SEQ ID NO:1] and/or Figure 3 [SEQ ID NO:3], or a fragment, analogue or derivative thereof.

In another particularly preferred embodiment of the present invention there is a novel sigma factor protein from Staphylococcus aureus comprising the amino acid sequence of Figure 2 [SEQ ID NO:2] and/or Figure [SEQ ID NO: 4], or a fragment, analogue or derivative thereof.

In accordance with this aspect of the present invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressible by the Staphylococcus aureus polynucleotide contained in NCIMB Deposit. No. 40771.

In accordance with this aspect of the invention there are provided isolated nucleic acid molecules encoding rsbU-1, particularly Staphylococcus rsbU-1, including mRNAs, cDNAs, genomic DNAs and, in further embodiments of this aspect of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically, useful variants, analogs or derivatives thereof, or fragments thereof, including fragments of the variants, analogs and derivatives, and compositions comprising same.

In accordance with another aspect of the present invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization.

Among the particularly preferred embodiments of this aspect of the invention are naturally occurring allelic variant of rsbU-1 and polypeptides encoded thereby.

In accordance with this aspect of the invention there are provided novel polypeptides of Staphylococcus referred to herein as rsbU-1 as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful fragments, variants and derivatives thereof, variants and derivatives of the fragments, and analogs of the foregoing, and compositions comprising same.

Among the particularly preferred embodiments of this aspect of the invention are variants of rsbU-1 polypeptide encoded by naturally occurring alleles of the rsbU-1 gene.

In a preferred embodiment of this aspect of the invention there are provided methods for producing the aforementioned rsbU-1 polypeptides.

In accordance with yet another aspect of the present invention, there are provided inhibitors to such polypeptides, useful as antibacterial agents, including, for example, antibodies.

In accordance with certain preferred embodinents of this aspect of the invention, there are provided products, compostions and methods, *inter alia:* assessing rsbU-1 expression; to treat upper respiratory tract (e.g. otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g. empyema, lung abscess), cardiac (e.g. infective endocarditis), gastrointestinal (e.g. secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g. cerebral abscess), eye (e.g. blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal & orbital cellulitis, darcryocystitis), kidney and urinary tract (e.g. epiditymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g. impetigo, folliculitis ; cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (eg. septic arthritis, osteomyelitis), assaying genetic variation, and administering a rsbU1 polypeptide or polynucleotide to an organism to raise an immunological response against a bacteria especially a Staphylococcus.

In accordance with certain preferred embodiments of this and other aspects of the invention there are polynucleotides that hybridize to rsbU-1 polynucleotide sequences.

In certain additional preferred embodiments of this aspect of the invention there are provided antibodies against rsbU-1 polypeptides.

In accordance with another aspect of the present invention, there are provided rsbU-1 agonists which are also preferably bacteriostatic or bacteriocidal.

In accordance with yet another aspect of the present invention, there are provided rsbU-1 antagonists which are also preferably bacteriostatic or bacteriocidal.

In a further aspect of the invention there are provided compositions comprising a rsbU-1 polynucleotide or a rsbU-1 polypeptide for administration to a cell or to a multicellular organism.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawgings depict certain embodiments of the invention. They are illustrative only and do not limit the invention otherwise diclosed herein.

Figure 1 shows the polynucleotide sequence of Staphylococcus aureus rsbU-1 [SEQ ID NO:1].

Figure 2 shows the amino acid sequence of Staphylococcus aureus rsbU-1 [SEQ ID NO:2] deduced from the polynucleotide sequence of Figure 1 [SEQ ID NO:1].

Figure 3 shows the polynucleotide sequence of Staphylococcus aureus rsbU-1 [SEQ ID NO:3].

Figure 4 shows the amino acid sequence of Staphylococcus aureus rsbU-1 [SEQ ID NO:4] deduced from the polynucleotide sequence of Figure 3 [SEQ ID NO:3].

Figure 5. shows the polynucleotide sequence of the sigB putative operon SEQ ID NO.5].

Figure 6. shows a bar diagram of the sigB putative operon structure including the relative orientations of rsbU-1, rsbV, rsbW and sigB.

GLOSSARY

The following illustrative explanations are provided to facilitate understanding of certain terms used frequently herein, particularly in the Examples. The explanations are provided as a convenience and are not limitative of the invention.

rsbU-1-BINDING MOLECULE, as used herein, refers to molecules or ions which bind or interact specifically with rsbU-1 polypeptides or polynucleotides of the present invention, including, for example enzyme substrates, cell membrane components and classical receptors. Binding between polypeptides of the invention and such molecules including binding or binding or interaction molecules may be exclusive to polypeptides of the invention, which is preferred, or it may be highly specific for polypeptides of the invention, which is also preferred, or it may be highly specific to a group of proteins that includes polypeptides of the invention, which is preferred, or it may be specific to several groups of proteins at least one of which includes a polypeptide of the invention. Binding molecules also include antibodies and antibody-derived reagents that bind specifically to polypeptides of the invention.

GENETIC ELEMENT generally means a polynucleotide comprising a region that encodes a polypeptide or a polynucleotide region that regulates replication, transcription or translation or other processes important to expression of the polypeptide in a host cell, or a polynucleotide comprising both a region that encodes a polypeptide and a region operably linked thereto that regulates expression. Genetic elements may be comprised within a vector that replicates as an episomal element, that is, as a molecule physically independent of the host cell genome. They may be comprised within plasmids. Genetic elements also may be comprised within a host cell genome; not in their natural state but, rather, following manipulation such as isolation, cloning and introduction into a host cell in the form of purified DNA or in a vector, among others.

HOST CELL is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polyncleotide sequence.

IDENTITY or SIMILARITY, as known in the art, are relationships between two polypeptide sequences or two polynucleotide sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between two polypeptide or two polynucleotide sequences as determined by the match between two strings of such sequences. Both identity and similarity can be readily calculated (*Computational Molecular Biology*, Lesk, A.M, ed., Oxford University Press, Net York, 1988; *Biocomputing: Informatics* and *Genome Projects*, Smith, D. W., ed., Academic Press, New York 1993; *Computer Analysis of Sequence Data,* Part I, Griffin, A.M., and Griffin. H. G, eds, Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer,* Gribskov, M. and Devereux, J., eds., M Stockton Press. New York, 1991). While there exist a number of methods to measure identity and similarity between two polynucleotide or two polypeptide sequences, both terms are well known to skilled artisans (*Sequence Analysis in Molecular Biology,* von Heinje, G., Academic Press, 1987; *Sequence Analysis Primer,* Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman D, SLAM *J. Applied Math., 48.* 1073 (1988). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM *.J. Applied Math., 48*: 1073(1988). Preferred methods to determine identity are designed to give the largest match between the two sequences tested. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., *Nucleic Acids Research 12(1)*: 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul. S.F. et al., *J. Molec. Biol. 215*:403 (1990)).

ISOLATED means altered "by the hand of man" from its natural state; *i.e.*, that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring polynucleotide or a polypeptide naturally present in a living organism in its natural state is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. For example, with respect to polynucleotides, the term isolated means that it is separated from the chromosome and cell in which it naturally occurs. As part of or following isolation, such polynucleotides can be joined to other polynucleotides, such as DNAs, for mutagenesis, to from fusion proteins, and for propagation or expression in a host, for instance. The isolated polynucleotides, alone or joined to other polynucleotides such as vectors, can be introduced into host cells; in culture or in whole organisms. Introduced into host cells in culture or in whole organisms, such DNAs still would be isolated, as the term is used herein, because they would not be in their naturally occuring form or environment. Similarly, the polynucleotides and polypeptides may occur in a composition, such as a media formulations,

solutions for introduction of polynucleotides or polypeptides. for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated polynucleotides or polypeptides within the meaning of that term as it is employed herein.

POLYNUCLEOTIDE(S) generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among others, single-and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double-and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded, or a mixture of single- and double-stranded regions. In addition polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of he molecules of a triple-helical region often is an oligonucleotide. As used herein, the term polynucleotide includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAS or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex, cells, *inter alia.* Polynucleotides embraces short polynucleotides often referred to as oligonucleotide(s).

POLYPEPTIDES, as used herein, includes all polypeptides as described below. The basic structure of polypeptides is well known and has been described in innumerable textboocks and other publications in the art. In this context, the term is used herein to refer to any peptide or protein comprising two or more amino acids joined to each other in a linear chain by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types; It will be appreciated that polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids. and that many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques which are well known to the art. Even the common modifications that occur naturally in polypeptides are too numerous to list exhaustively here, but they are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. Among the known modifications which may be present in polypeptides of the present are, to name an illustrative few, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment. sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance *PROTEINS-STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed, T. E. Creighton, W. H. Freeman and Company. New York (1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold. F., Posttranslational Protein Modifications: Perspectives and Prospects. pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS,* B.C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging*. Ann. N.Y. Acad. Sci. 663: 48-62(1992). It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular. with or without branching, generally as a result of posttranslation events. including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translation natural process and by entirely synthetic methods, as well. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact. blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occuring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention. as well. For instance. the amino terminal residue of polypeptides made in *E. coli* or other cells, prior to proteolytic processing, almost invariably will be N-formylmethionine. During post-trans-

lational modification of the peptide, a methionine residue at the NH_2-terminus may be deleted. Accordingly, this invention contemplates the use of both the methionine-containing and the methionineless amino terminal variants of the protein of the invention. The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extend of the modifications in large part will be determined by the host cell posttranslational modification capacity and the modification signals present in the polypeptide amino acid sequence. For instance, as is well known, glycosylation often does not occur in bacterial host such as, for example, *E. coli*. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell. Insect cell often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been developed to express efficiently mammalian proteins having native patterns of glycosylation, *inter alia*. Similar considerations apply to other modifications. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized recombinantly by expressing a polynucleotide in a host cell.

VARIANT(S) of polynucleotides or polypeptides, as the term is used herein, are polynucleotides or polypeptides that differ from a reference polynucleotide or polypeptide, respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail. (1) A polynucleotide that differs in nucleotide sequence from another, reference polynucleotide. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical. As noted below, changes in the nucleotide sequence of the variant may be silent. That is, they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference. Also as noted below, changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. (2) A polypeptide that differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many region, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel rsbU-1 polypeptides and polynucleotides, among other things, as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of a novel rsbU-1 gene of Staphylococcus aureus, which is related by amino acid sequence homology to in_homo_gensp Bacillus subtilis rsbU polypeptide. The invention relates especially to rsbU-1 having the amino acid sequences set out in Figures 1 and 3, and the nucleic acid sequences set out in Figures 2, 4 and 5, and to the rsbU-1 nucleotide and amino acid sequences of the DNA in NCIMB Deposit No.40771, which is herein referred to as "the deposited clone" or as the "DNA of the deposited clone" It will be appreciated that the nucleotide and amino acid sequences set out in Figures I [SEQ ID NO: 1] and 2 [SEQ ID NO:2] where obtained by sequencing the DNA of the deposited clone. Hence, the sequence of the deposited clone is controlling as to any discrepancies between it (and the sequence it encodes) and the sequences of Figure 1 [SEQ ID NO: 1] and Figure 2 [SEQ ID NO:2], or Figure 3 [SEQ ID NO:3] and Figure 4 [SEQ ID NO :4].

Polynucleotides

In accordance with one aspect of the present invention, there are provided isolated polynucleotides which encode the rsbU-1 polypeptide having the deduced amino acid sequence of

Figure 2 [SEQ ID NO:2] and/or Figure [SEQ ID NO:4]. Provided are two different GTG initiation codon (methionine) start sites for the gene provide herein

Using the information provided herein, such as the polynucleotide sequence set out in Figure 1 [SEQ ID NO:1] and/or Figure 3 [SEQ ID NO:3] and/or Figure 5 [SEQ ID NO:5], a polynucleotide of the present invention encoding rsbU-1 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning and sequencing chromosomal DNA fragments from Staphylococcus aureus WCUH29 cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide of the invention sequence, such as that sequence given in Figure 1 [SEQ ID NO:1] and/or Figure 3 [SEQ ID NO:3] and/or Figure 5 [SEQ ID NO:5] typically a library of clones of chromosomal DNA of Staphylococcus aureus WCUH29 in *E.coli* or some other suitable host is probed with a radiolabelled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using high stringency washes. By sequencing the individual clones thus identified with sequencing primers designed from the original sequence it is then possible

to extend the sequence in both directions to determine the full gene sequence. Conveniently such sequencing is performed using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch. E. F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13. 70). Illustrative of the invention, the polynucleotides set out in Figure 1 [SEQ ID NO:1], Figure 3 [SEQ ID NO:3] and Figure 5 [SEQ ID NO :5] were discovered in a DNA library derived from Staphylococcus aureus WCUH29.

rsbU-1 of the invention is structurally related to other proteins of the SigB operon family, as shown by the results of sequencing the DNA encoding rsbU-1 of the deposited clone. The DNA sequences thus obtained are set out in Figure 1 [SEQ ID NO:1], Figure 3 [SEQ ID NO:3], and Figure 5 [SEQ ID NO:5]. It contains an open reading frame encoding a protein of having about the number of amino acid residues set forth in Figure 2 [SEQ ID NO:2] and Figure 4 [SEQ ID NO:4] respectively with deduced molecular weights that can be calculated using amino acid residue molecular weight values well known in the art. The protein exhibits greatest homology to Bacillus subtilis rsbU protein among known proteins. rsbU-1 of Figure 2 [SEQ ID NO:2] has about 39.1% identity over its entire length and about 62.1% similarity over its entire length with the amino acid sequence of Bacillus subtilis rsbU.

Polynucleotides of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be double stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The coding sequence which encodes the polypeptide may be identical to the coding sequence of the polynucleotide shown in Figure 1 [SEQ ID NO:1] and/or Figure 3 [SEQ ID NO:3] and/or Figure 5 [SEQ ID NO:5]. It also may be a polynucleotide with a different sequence, which, as a result of the redundancy (degeneracy) of the genetic code, encodes the polypeptide of Figure 2 [SEQ ID NO:2] and/or Figure 4 [SEQ ID NO:4]

Polynucleotides of the present invention which encode the polypeptide of Figure 2 [SEQ ID NO:2] and/or Figure 4 [SEQ ID NO:4] may include, but are not limited to the coding sequence for the mature polypeptitde, by itself, the coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence, the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription (including termination signals, for example), ribosome binding, mRNA stability elements, and additional coding sequence which encode additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain embodiments of this aspect of the invention, the maker sequence is a hexa-histidine peptide, such as the tag provided in the pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz et al. *Proc. Natl. Acad. Sci., USA 86*. 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The HA tag may also be used to create fusion proteins and corresponds to an epitope derived of influenza hemaglutinin protein, which has been described by Wilson *et al., Cell 37*. 767 (1984), for instance. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated genetic elements.

In accordance with the foregoing, the term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides which include a sequence encoding a polypeptide of the present invention, particularly bacterial, and more particularly the Staphylococcus aureus rsbU-1 having the amino acid sequence set out in Figure 2 [SEQ ID NO: 2] or Figure 4 [SEQ ID NO:4]. The term encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by integrated phage or insertion sequence or editing) together with additional regions, that also may contain coding and/or noncoding sequences

The present invention further relates to variants of the herein above described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 2 [SEQ ID NO.2] and/or Figure 4 [SEQ ID NO.4]. A variant of the polynucleotide may be a naturally occuring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naurally occuring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms.

Among variants in this regard are variant that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or noncoding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions.

Among the particularly preferred embodiments of the invention in this regard are polynucleotides encoding polypeptides having the amino acid sequence of rsbU-1 set out in Figure 2[SEQ ID NO:2] and/or Figure 4 [SEQ ID NO:4]; variants, analogs, derivatives and fragments thereof, and fragments of the variants, analogs and derivatives.

Further particularly preferred in this regard are polynucleotides encoding rsbU-1 variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which have the amino acid sequence of rsbU-1 polypeptide of Figure 2 [SEQ ID NO:2] and/or Figure 4 [SEQ ID NO:4] in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of rsbU-1. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polynucleotides encoding polypeptides having the amino acid sequence of Figure 2 [SEQ ID NO:2] and/or Figure 4 [SEQ ID NO:4], without substitutions.

Further preferred embodiment of the invention are polynucleotides that are at least 70% identical over their entire length to a polynucleotide encoding rsbU-1 polypeptide having the amino acid sequence set out in Figure 2 [SEQ ID NO:2] and/or Figure 4 [SEQ ID NO:4], and polynucleotides which are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over their entire length to a polynucleotide encoding rsbU-1 polypeptide of the Staphylococcus aureus DNA of the deposited clone and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments in this respect, moreover, are polynucleotides which encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the DNA of Figure 1 [SEQ ID NO:1] and/or Figure 3 [SEQ ID NO:3] and/or Figure 5 [SEQ ID NO:5].

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding rsbU-1 and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the rsbU-1 gene. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases. Particularly, preferred probes will have at least 30 bases and will have 50 bases or less.

For example, the coding region of the rsbU-1 gene may be isolated by screening using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the present invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe, hybridizes to.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to polynucleotide assays, *inter alia*.

The polynucleotides of the invention that are oligonucleotides derived from the sequences of SEQ ID NOS: 1 and 2 may be used as PCR primers in the process herein described to determine whether or not the Staphylococcus aureus genes identified herein in whole or in part are transcribed in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained

The polynucleotides may encode a polypeptide which is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form may allow protein transport, may lengthen or shorten protein half-life or many facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In sum, a polynucleotide of the present invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences which are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

Deposited materials

The deposit has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposit is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C §112.

A deposit containing a Staphylococcus aureus rsbU-1 bacterial clone has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. (NCIMB). 23 St. Machar Drive, Aberdeen AB2 IRY, Scotland on 11 September 1995 and assigned NCIMB Deposit No.40771. The Staphylococcus aureus bacterial clone deposit it referred to herein as "the deposited clone" or as "the DNA, of the deposited clone."

The deposited material is a bacterial clone that contains the full length rsbU-1 DNA, referred to as "Staphylococus areus WCUH29" upon deposit.

The sequence of the polynucleotides contained in the deposited material, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

Polypeptides

The present invention further relates to a rsbU-1 polypeptide which has a deduced amino acid sequence of 333 or 339 amino acids in lenght, as set forth in Figure 2 [SEQ ID NO: 2] and/or Figure 4 [SEQ ID NO:4] respectively.

The invention also relates to fragments, analogs and derivates of these polypeptides. The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 2 [SEQ ID NO:2], means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The fragment derivative or analog of the polypeptide of Figure 2 [SEQ ID NO:2] may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Among the particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of rsbU-1 set out in Figure 2 [SEQ ID NO:2], variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments. Alternatively, particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of the rsbU-1, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, having the amino acid sequence of the rsbU-1 polypeptide of Figure 2 [SEQ ID NO: 2], in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the rsbU-1. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polypeptides having the amino acid sequence of Figure 2 [SEQ ID NO:2] without substitutions.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity

The polypeptides of the present invention include the polypeptide of Figure 2 [SEQ ID NO:2] (in particular the mature polypeptide) as well as polypeptides which have at least 70% identity to the polypeptide of Figure 2 [SEQ ID NO:2], preferably at least 80% identity to the polypeptide of Figure 2 [SEQ ID NO:2], and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide of Figure 2 [SEQ ID NO:2] and still more preferably at least 95°% similarity (still more preferably at least 95% identity) to the polypeptide of Figure 2 [SEQ ID NO:2] and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino

acids and more preferably at least 50 amino acids.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

Fragments

Also among preferred embodiments of this aspect of the present invention are polypeptides comprising fragments of rsbU-1, most particularly fragments of rsbU-1 having the amino acid set out in Figure 2[SEQ ID NO:2], and fragments of variants and derivatives of the rsbU-1 of Figure 2 [SEQ ID NO:2].

In this regard a fragment is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned rsbU-1 polypeptides and variants or derivatives thereof.

Such fragments may be "free-standing," *i.e.*, not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the presently discussed fragments most preferably form a single continuous region. However, several fragments may be comprised within a single larger polypeptide. For instance, certain preferred embodiments relate to a fragment of a rsbU-1 polypeptide of the present comprised within a precursor polypeptide designed for expression in a host and having heterologous pre and pro-polypeptide regions fused to the amino terminus of the rsbU-1 fragment and an additional region fused to the carboxyl terminus of the fragment. Therefore, fragments in one aspect of the meaning intended herein, refers to the portion or portions of a fusion polypeptide or fusion protein derived from rsbU-1.

Representative examples of polypeptide fragments of the invention, include, for example, fragments from amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101-333, and any combination of these 20 amino acid fragments.

In this context "about" herein includes the particularly recited ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments of the invention include, for example, truncation polypeptides of rsbU-1. Truncation polypeptides include rsbU-1 polypeptides having the amino acid sequence of Figure 2, or of variants or derivatives thereof, except for deletion of a continuous series of residues (that is, a continuous region, part or portion) that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or, as in double truncation mutants, deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Fragments having the size ranges set out about also are preferred embodiments of truncation fragments, which are especially preferred among fragments generally. Degradation forms of the polypeptides of the invention in a host cell, particularly a Staphylococcus, are also preferred.

Also preferred in this aspect of the invention are fragments characterized by structural or functional attributes of rsbU-1. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions of rsbU-1, and combinations of such fragments.

Preferred regions are those that mediate activities of rsbU-1. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of rsbU-1, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Further preferred polypeptide fragments are those that are antigenic or immunogenic in an animal, especially in a human.

It will be appreciated that the invention also relates to, among others, polynucleotides encoding the aforementioned fragments, polynucleotides that hybridize to polynucleotides encoding the fragments, particularly those that hybridize under stringent conditions, and polynucleotides, such as PCR primers, for amplifying polynucleotides that encode the fragments. In these regards, preferred polynucleotides are those that correspondent to the preferred fragments, as discussed above.

Vectors, bost cells, expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention. Introduction of a polynucleotides into the host cell can be affected by calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction, infection or other methods. Such methods are described in many standard

laboratory manuals, such as Davis et al. *BASIC METHODS IN MOLECULAR BIOLOGY,* (1986) and Sambrook et al, *MOLECULAR CLONING : A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.(1989).

Polynucelotide constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derivated from the DNA construcls of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., *MOLECULAR CLONING : A LABORATORY MANUAL*, 2nd Ed., Cold Spfing Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Plasmids generally are designated herein by a lower case p preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art. Starting plasmids disclosed herein are either commercially available, publicly available, or can be constructed from available plasmids by routine application of well known, published procedures. Many plasmids and other cloning and expression vectors that can be used in accordance with the present invention are well known and readily available to those of skill in the art.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise *cis*-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate *trans*-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

In certain preferred embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those of skill in the art.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include, among others chromosomal, episomal and virus-derived vectors, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al., MOLECULAR CLONING, A LABORATORY MANUAL,* 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s), including, for instance, a promoter to direct mRNA transcription Representatives of such promoters include, but are not limited to, the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs.

In general, expression constructs will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, in accordance with many commonly practiced procedures, such regions will operate by controlling transcription, such as transcription factors, repressor binding sites and termination, among others.

Vectors for propagation and expression generally will include selectable markers and amplification regions, such as, for example, those set forth in Sambrook *et al., MOLECULAR CLONING, A LABORATORY MANUAL,* 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

Representative examples of appropriate hosts include bacterial cells, such as streptocooi, staphylococci, *E. coli*, streptomyces and *Salmonella typhimurium* cells fungal cells, such as yeast cells and *Aspergillus* cells; insect cells, such as *Drosophila* S2 and *Spodoptera* Sf9 cells: animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells: and plant cells.

The following vectors, which are commercially available, are provided by way of example. Among vectors preferred

for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A. available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia, and pBR322 (ATCC 37017). Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL, available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase ("CAT") transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment *i.e.*, a fragment that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the *cat* gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available, such as pKK232-8 and pCM7. Promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene.

Among known prokaryotic promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the *E. coli* lacI and lacZ promoters, the T3 and T7 promoters, the *gpr* promoter, the lambda PR, PL promoters and the trp promoter.

Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Recombinant expression vectors will include, for example, origins of replication, a promoter preferably derived from a highly-expressed gene to direct transcription of a downstream stuctural sequence, and a selectable marker to permit isolation of vector containing cells after exposure to the vector.

Polynucleotides of the invention, encoding the heterologous structural sequence of a polypeptide of the invention generally will be inserted into the vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site is located appropriately 5' to a ribosome binding site. The ribosome binding site will be 5' to the AUG that initiates translation of the polypeptide to be expressed. Generally, there will be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiation codon. Also, generally, there will be a translation stop codon at the end of the polypeptide and there will be a polyadenylation signal in constructs for use in eukaryotic hosts. Transcription termination signal appropriately disposed at the 3' end of the transcribed region may also be included in the polynucleotide construct.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the C- or N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability or to facilitate purification, among others, are familiar and routine techniques in the art A preferred fusion protein comprises a heterologous region from immunolglobulin that is useful to solubilize or purify polypeptides. For example, EP-A-0 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another protein or part thereof. In drug discovery, for example, proteins have been fused with antibody Fc portions for the purpose of high-throughput screening assays to identify antagonists.See, D. Bennett *et al.*, *Journal of Molecular Recognition*, Vol. 8 52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry*, Vol 270, No. 16, pp 9459-9471 (1995).

Cells typically then are harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Mammalian expression vectors may comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation regions, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences that are necessary for expression.

rsbU-1 polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Polynucleotide assays

This invention is also related to the use of the rsbU-1 polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of rsbU-1 in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of a disease. Eukaryotes (herein also "individual(s), particularly mammals, and especially humans, infected with an organism comprisng the rsbU-1 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from an infected individual's cells and tissues, such as bone, blood, muscle, cartilage, and skin. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki et al., *Nature, 324*, 163-166 (1986) prior to analysis RNA or cDNA may also to be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding rsbU-1 can be used to identify and analyze rsbU-1 presence and/or expression. Using PCR characterization of the strain of prokaryote present in a eukaryote, particularly a mammal, and especially a human, may be made by an analysis of the genotype of the prokaryote gene. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the genotype of a reference sequence. Point mutations can be identified by hybridizing amplified DNA to radiolabeled rsbU-1 RNA or alternatively, radiolabeled rsbU-1 antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic characterization based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, *e.g.*, Myers et al., *Science, 230:* 1242(1985)).

Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (*e.g.*, Cotton et al., *Proc. Natl. Acad. Sci., USA, 85:* 4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection chemical cleavage, direct DNA sequencing or the use of restriction enzymes, *e.g.*, restriction fragment length polymorphisms (RFLP) and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing mutations also can be detected by *in situ* analysis.

Cells carrying mutations or polymorphisms in the gene of the present invention may also be detected at the DNA level by a variety of techniques to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations. It is particularly preferred to used RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA or cDNA may also be used for the same purpose, PCR or RT-PCR. As an example, PCR primers complementary to the nucleic acid encoding rsbU-1 can be made using known methods and used to identify and analyze mutations. These primers may be used or amplifying rsbU-1 DNA isolated from a sample derived from an individual. The invention also provides these primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. The primers may be used to amplify the gene isolated from the individual such that the gene may then be subject to various techniques for elucidation of the DNA sequence. In this way, mutations in the DNA sequence may be diagnosed.

The invention provides a process for diagnosing, disease, preferably bacterial infections, more preferably Staphylococcus aureus, and most preferably upper respiratory tract (e.g. otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g. empyema, lung abscess), cardiac (e.g infective endocarditis), gastrointestinal (e.g. secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g. cerebral abscess), eye (e.g. blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal & orbital cellulitis, darcryocystitis), kidney and urinary tract (e.g.

epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g. impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g. septic arthritis, osteomyelitis), comprising determining from a sample derived from an individual a increased level of expression of polynucleotide having the sequence of Figure 1 [SEQ ID NO:1] and/or Figure 3 [SEQ ID NO:3] and/or Figure 5 [SEQ ID NO:5]. Increased expression of rsbU-1 polynucleotide can be measured using any on of the methods well known in the art for the quantation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

Polypeptide assays

The present invention also relates to a diagnostic assays such as quantitative and diagnostic assays for detecting levels of rsbU-1 protein in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of rsbU-1 protein compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a rsbU-1 protein, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blor analysis and ELISA assays. Among these ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to rsbU-1, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached a detectable reagent such as radioactive, fluorescent or enzymatic reagent in this example horseradish peroxidase enzyme.

Antibodies

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. The present invention includes, for examples monoclonal and polyclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique known in the art which provides antibodies produced by continuous cell line cultures can be used. Examples include various technique, suchas those in Kohler, G. and Milstein C., *Nature 256:* 495-497 (1975); Kozbor *et al., Immunology Today 4:* 72 (1983); Cole et al., pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

Alternatively phage display technology could be utilised to select antibody genes with binding activities towards the polypeptide either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-Fbp or from naive libraries (McCafferty, J. et al., (1990), Nature **348**, 552-554, Marks, J. et al. (1992) Biotechnology *10*, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T et al., (1991) Nature **352**, 624-628).

If two antigen binding domains are present each domain may be directed against a different epitope - termed 'bispecific' antibodies.

The above-described antibodies may be employed to isolate or to identity clones expressing the polypeptide or purify the polypeptide of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

Thus, among others, antibodies against rsbU-1 may be employed to inhibit and/or treat infections, particularly bacterial infections and especially upper respiratory tract (e.g. otitis media, bacterial tracheitis, acute epiglottitis, thyroïditis), lower respiratory (e.g. empyema, lung abscess),cardiac (e.g. infective endocarditis), gastrointestinal (e.g. secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (eg. cerebral abscess), eye (e.g. blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal & orbital cellulitis, darcryocystitis), kidney and urinary tract (e.g. epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g. impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g. septic arthritis, osteomyelitis).

Polypeptide derivatives include antigenically, epitopically or immunologically equivalent derivatives which form a

particular aspect of this invention. The term "antigenically equivalent derivative" as used herin encompasses a polypeptide or its equivalent which will be specifically recognised by certain antibodies which, when raised to the protein or polypeptide according to the present invention, interfere with the immediate physical interaction between pathogen and mammalian host, directly or indirectly. The term "immunologically equivalent derivative" as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the immediate physical interaction between pathogen and mammalian host, directly or indirectly.

The polypeptide, such as an antigenically or immunologically equivalent derivative or a fusion protein thereof is used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the polypeptide. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively a multiple antigenic peptide comprising multiple copies of the the protein or polypeptide, or an antigenically or immunologically equivalent polypeptide thereof may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

Preferably the antibody or derivative thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanised"; where the complimentarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones, P. et al (1986), Nature **321**, 522-525 or Tempest et al., (1991) Biotechnology 9, 266-273.

The use of a polynucleotide of the invention in genetic immunisation will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff et al., Hum Mol Genet 1992, 1-363, Manthorpe et al., Hum. Gene Ther. 1963:4, 419), delivery of DNA complexed with specific protein carriers (Wu et al., J Biol Chem 1989:264, 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, PNAS,1986:83,9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., Science 1989:243,375), particle bombardment (Tang et al., Nature 1992, 356:152, Eisenbraun et al., DNA Cell Biol 1993, 12:791) and *in vivo* infection using cloned retroviral vectors (Seeger et al., PNAS 1984:81,5849).

rsbU-1-binding molecules and assays

This invention also provides a method for identification of molecules, such as binding molecules, that bind rsbU-1. Genes encoding proteins that bind rsbU-1, can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Such methods are described in many laboratory manuals such as, for instance, Coligan *et al., Current Protocols in Immunology 1(2):* Chapter 5 (1991). Also, a labeled ligand can be photoaffinity linked to a cell extract. Polypeptides of the invention also can be used to assess rsbU-1 binding capacity of rsbU-1-binding molecules, in cells or in cell-free preparations.

Polypeptides of the invention may also be used to assess the binding or small molecule substrates and ligands in, for example, cells-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics.

Antagonists and Agonists - assays and molecules

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of rsbU-1 polypeptides or polynucleotides, such as its interaction with rsbU-1-binding molecules.

For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, may be prepared from a cell that expresses a molecule that binds rsbU-1. The preparation is incubated with labeled rsbU-1 in the absence or the presence of a candidate molecule which may be a rsbU-1 agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, *i.e.*, without inducing the effects of rsbU-1 on binding the rsbU-1 binding molecule, are most likely to be good antagonists. Molecules that bind well and elicit affects that are the same as or closely related to rsbU-1 are agonists.

rsbU-1-like effects of potential agonists and antagonists may by measured, for instance, by determining activity of a reporter system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of rsbU-1 or molecules that elicit the same effects as rsbU-1. Reporter systems that may be useful in this regard include but are not limited to colorimetic labeled substrate converted into product, a reporter gene that is responsive to changes in rsbU-1 activity, and binding assays known in the art

Another example of an assay for rsbU-1 antagonists is a competitive assay that combines rsbU-1 and a potential antagonist with membrane-bound rsbU-1-binding molecules, recombinant rsbU-1 binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. rsbU-1 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of rsbU-1 molecules bound to a binding mulocule or converted to product can be determined accurately to assess the effectiveness of the potential

antagonist.

Potential antagonists include small molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing rsbU-1-induced activities, thereby preventing the action of rsbU-1 by excluding rsbU-1 from binding.

Potential antagonists include a small molecule which binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules.

Other potential antagonists include antisense molecules (see *Okano, J. Neurochem, 56:* 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL(1988), for a description of these molecules).

Preferred potential antagonists include compounds related to and derivatives of rsbU-1.

In a particular aspect the invention provides the use of the polypeptide, polynucleotide or inhibitor of the invention to interfere, directly or indirectly, with the initial physical interaction between a pathogen and mammalian host responsible for sequelae of infection. In particular the molecules of the invention may be used: i) in the prevention of adhesion of bacteria, in particular gram positive bacteria, to mammalian extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; ii) to block sigma factor protein mediated mammalian cell invasion by, for example, initiating phosphorylation of mammalian tyrosine kinases (Rosenshine *et al., Infect. Immun, 60:*2211 (1992); iii) to block bacterial adhesion between mammalian extracellular matrix proteins and bacterial sigma factor proteins which mediate tissue damage; iv) to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

Each of the DNA sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein upon expression call be used as a target for the screening of antibacterial drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgamo or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The antagonists and agonists may be employed for instance to inhibit upper respiratory tract (e.g. otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g. empyema, lung abscess),cardiac (e.g. infective endocarditis), gastrointestinal (e.g. secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g. cerebral abscess), eye (e.g. blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal & orbital cellulitis, darcryocystitis), kidney and urinary tract (e.g. epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g. impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g. septic arthritis, ostecmyelitis).

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal which comprises inoculating the individual with rsbU-1, or a fragment or variant thereof, adequate to produce antibody to protect said individual from infection, particularly bacterial infection and most particularly Staphylococcus infections. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises, through gene therapy, delivering gene encoding rsbU-1, or a fragment or a variant thereof, for expressing rsbU-1, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibody to protect said individual from disease.

A further aspect of the invention relates to an immunological composition which, when introduced into a host capable or having iduced within it an immunological response, induces an immunological response in such host to a rsbU-1 or protein coded therefrom, wherein the composition comprises a recombinant rsbU-1 or protein coded therefrom comprising DNA which codes for and expresses an antigen of said rsbU-1 or protein coded therefrom.

The rsbU-1 or a fragment thereof may be fused with co-protein which may not by itself produce antibodies, but is capable of stabilizing the first protein and producing a fused protein which will have immunogenic and protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as Glutathione-S-transferase (GST) or beta-galactosidase, relatively large co-proteins which solubilise the protein and facilitate production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the fist protein

Provided by this invention are methods using the described polynucleotide or particular fragments thereof which have been shown to encode non-variable regions of bacterial cell surface proteins in DNA constructs used in such genetic immunization experiments in animal models of infection with Staphylococcus aureus will be particularly useful

for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approch will allow for the subsequent preparation of monoclonal antibodies of particular value from the requisite organ of the animal successfully resisting or clearing infection for the development of prophylatic agents or therapeutic treatments of Staphylococcus aureus infection in mammals, particularly humans.

The polypeptide may be used as an antigen for vaccination of a host ro produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused e.g. by mechanical, chemical or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, mammary glands, urethra or vagina.

The present invention also includes a vaccine formulation which comprises the immunogenic recombinant protein together with a suitable carrier. Since the protein may be broken down in the stomach, it is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the bodily fluid, preferably the blood, of the individual, and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

While the invention has been described with reference to certain rsbU-1, it is to be understood that this covers fragments of the naturally occurring protein and similar proteins with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant protein.

Compositions

The invention also relates to compositions comprising the polynucleotide or the polypeptides discussed above or the agonists or antagonists. Thus, the polypeptides of the present invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

Kits

The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture, use or sale of the product for human administration.

Administration

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

The pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific indication or indications. In general, the compositions are administered in an amount of at least about 10 μg/kg body weight. In most cases they will be administered in an amount not in excess of about 8 mg/kg body weight per day. Preferably, in most cases, dose is from about 10 μg/kg to about 1 mg/kg body weight, daily. It will be appreciated that optimum dosage will be determined by standard methods for each treatment modality and indication, taking into account the indication, its severity, route of administration, complicating conditions and the like

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application for example in the form of ointments, creams,

lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation: more usually they will constitute up to about 80% by weight of the formulation.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mgkg, tipically around 1 mg/kg. The physician in any event will determine the actual dosase which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In-dwelling devices include surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include , for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shuns, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters, etc.

The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent Staphylococcus wound infections.

Many orthopaedic surgeons consider that humans with prosthetic joint should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteraemia. Late deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an elternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1 µg/ml to 10mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response.

A suitable unit dose for vaccination is 0.5-5µg/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks.

With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

The antibodies described above may also be used as diagnostic reagents to detect the presence of bacteria containing the sigma factor protein.

In order to facilitate understanding of the following example certain frequently occuring methods and/or terms will be described.

EXAMPLES

The present invention is further described by the following examples. These exemplification's, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

Certain terms used herein are explained in the foregoing glossary.

All examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Example 1 Library Production

The polynucleotide having the DNA sequence given in SEQ ID NO:1 was obtained from a library of clones of chromosomal DNA of Staphylococcus aureus in E. coli. In some cases the sequencing data from two or more clones containing overlapping Staphylococcus aureus DNAs was used to construct the contiguous DNA sequence in SEQ ID NO:1. Libraries may be prepared by routine methods, for example.

Methods 1 and 2 below.

Total cellular DNA is isolated from Staphylococcus aureus WCUH29 according to standard procedures and size-fractionted by either of two methods.

Method 1

Total cellular DNA is mechanically sheared by passage through a needle in order to size-fractionate according to standard procedures. DNA fragments of up to 1 lkbp in size are rendered blunt by treatment with exonuclease and DNA polymerase, and EcoRI linkers added. Fragments are ligated into the vector Lambda ZapII that has been cut with EcoRI, the library packaged by standard procedures and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

Method 2

Total cellular DNA is partially hydrolsed with a combination of four restriction enzymes (RsaI, PallI, AluI and Bsh1235I) and size-fractionated according to standard procedures. EcoRI linkers are ligated to the DNA and the fragments then ligated into the vector Lambda ZapII that have been cut with EcoRI, the library packaged by standard procedures, and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT
(A) NAME: SmithKline Beecham Corporation
(B) STREET: One Franklin Plaza
(C) CITY: Philadelphia
(D) STATE OR PROVINCE: PA
(E) COUNTRY: USA
(F) POSTAL CODE: 19103

(ii) TITLE OF THE INVENTION: Novel rsbU-1

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER-READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1002 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTGGAAGAAT TTAAGCAACA TTATAAGGGT TTAATTGATG AAAGTTTAAC
GTGCCAAGAT 60

AAAGTAGAAT TGATAAAAAA GTGTGAGAAA TACACTGACG AAGTGATTCG TAAGGACGTC 120
TTGCCTGAAG ACATTGTCGA TATTCACAAA AACTATATAT TGACGTTAAA CTTAACGCGT 180
GAAGATGTGT TCAAGACATT AGATGTCTTA CAAGAAATCG TTAAAGGCTT TGGTTATAGT 240
TATCGAGATT ATCAAAGATT GGTAGATAAA CTTCAAGTTC ACGATAAAGA GATAGACTTA 300
GCTTCTAGCT TACAACCAAC AATGCTTAAA ACAGATATTC CACAATTTGA TAGTATTCAA 360
ATTGGCGTTA TTTCAGTGGC AGCACAAAAA GTAAGTGGAG ATTATTTTAA TTTAATTGAC 420
CATAACGATG GCACAATGAG CTTTGCTGTT GCAGATGTCA TTGGGAAAGG TATACCAGCT 480
GCTTTAGCAA TGAGTATGAT AAAGTTTGGC ATGGATTCTT ATGGACACTC ACAATTACCG 540
AGTGATGGCT TAAAACGTTT AAATCGTGTT GTTGAAAAGA ATATTAATCA AAATATGTTC 600
GTCACAATGT TTTATGGTTT ATATGAAGAA ATGAACCATT TATTGTATTG TAGTTCAGCT 660
GGTCATGAGC CTGGATATAT TTATCGCGCT GAAAAAGAAG AATTTGAAGA AATTTCAGTT 720
AGAGGTAGAG TGTTAGGAAT CAGTTCACAA ACACGATATC AACAACAAGA AATTCCTATA 780
TACCTTGATG ATTTAATTAT CATTTTAACG GATGGTGTGA CTGAAGCTAG AAATAGTGAA 840
GGTACCTTTA TAGATAAACA AAAACTTTTA GAATATATTA AAAAACATAA ACATATGCAC 900
CCACAAGATA TTGTTCAAAT TATCTATGAA GCAATTTTAA AGCTTCAAAA CCCAAATAAA 960
AAAGATGATA TGACTATTTT GATTATAAAA AGAGTAAATT AA 1002

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Glu Phe Lys Gln His Tyr Lys Gly Leu Ile Asp Glu Ser Leu
1 5 10 15
Thr Cys Gln Asp Lys Val Glu Leu Ile Lys Lys Cys Glu Lys Tyr Thr
20 25 30
Asp Glu Val Ile Arg Lys Asp Val Leu Pro Glu Asp Ile Val Asp Ile
35 40 45
His Lys Asn Tyr Ile Leu Thr Leu Asn Leu Thr Arg Glu Asp Val Phe
50 55 60
Lys Thr Leu Asp Val Leu Gln Glu Ile Val Lys Gly Phe Gly Tyr Ser
65 70 75 80
Tyr Arg Asp Tyr Gln Arg Leu Val Asp Lys Leu Gln Val His Asp Lys
85 90 95
Glu Ile Asp Leu Ala Ser Ser Leu Gln Pro Thr Met Leu Lys Thr Asp
100 105 110
Ile Pro Gln Phe Asp Ser Ile Gln Ile Gly Val Ile Ser Val Ala Ala
115 120 125
Gln Lys Val Ser Gly Asp Tyr Phe Asn Leu Ile Asp His Asn Asp Gly
130 135 140
Thr Met Ser Phe Ala Val Ala Asp Val Ile Gly Lys Gly Ile Pro Ala
145 150 155 160
Ala Leu Ala Met Ser Met Ile Lys Phe Gly Met Asp Ser Tyr Gly His
165 170 175
Ser Gln Leu Pro Ser Asp Gly Leu Lys Arg Leu Asn Arg Val Val Glu
180 185 190
Lys Asn Ile Asn Gln Asn Met Phe Val Thr Met Phe Tyr Gly Leu Tyr
195 200 205
Glu Glu Met Asn His Leu Leu Tyr Cys Ser Ser Ala Gly His Glu Pro
210 215 220
Gly Tyr Ile Tyr Arg Ala Glu Lys Glu Glu Phe Glu Glu Ile Ser Val
225 230 235 240
Arg Gly Arg Val Leu Gly Ile Ser Ser Gln Thr Arg Tyr Gln Gln Gln
245 250 255
Glu Ile Pro Ile Tyr Leu Asp Asp Leu Ile Ile Ile Leu Thr Asp Gly
260 265 270
Val Thr Glu Ala Arg Asn Ser Glu Gly Thr Phe Ile Asp Lys Gln Lys
275 280 285
Leu Leu Glu Tyr Ile Lys Lys His Lys His Met His Pro Gln Asp Ile
290 295 300
Val Gln Ile Ile Tyr Glu Ala Ile Leu Lys Leu Gln Asn Pro Asn Lys
305 310 315 320
Lys Asp Asp Met Thr Ile Leu Ile Ile Lys Arg Val Asn

325 330

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1020 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTGAGGAGGC AACTAATCGT GGAAGAATTT AAGCAACATT ATAAGGGTTT AATTGATGAA 60
AGTTTAACGT GCCAAGATAA AGTAGAATTG ATAAAAAAGT GTGAGAAATA CACTGACGAA 120
GTGATTCGTA AGGACGTCTT GCCTGAAGAC ATTGTCGATA TTCACAAAAA CTATATATTG 180
ACGTTAAACT TAACGCGTGA AGATGTGTTC AAGACATTAG ATGTCTTACA AGAAATCGTT 240
AAAGGCTTTG GTTATAGTTA TCGAGATTAT CAAAGATTGG TAGATAAACT TCAAGTTCAC 300
GATAAAGAGA TAGACTTAGC TTCTAGCTTA CAACCAACAA TGCTTAAAAC AGATATTCCA 360
CAATTTGATA GTATTCAAAT TGGCGTTATT TCAGTGGCAG CACAAAAAGT AAGTGGAGAT 420
TATTTTAATT TAATTGACCA TAACGATGGC ACAATGAGCT TTGCTGTTGC AGATGTCATT 480
GGGAAAGGTA TACCAGCTGC TTTAGCAATG AGTATGATAA AGTTTGGCAT GGATTCTTAT 540
GGACACTCAC AATTACCGAG TGATGGCTTA AAACGTTTAA ATCGTGTTGT TGAAAAGAAT 600
ATTAATCAAA ATATGTTCGT CACAATGTTT TATGGTTTAT ATGAAGAAAT GAACCATTTA 660
TTGTATTGTA GTTCAGCTGG TCATGAGCCT GGATATATTT ATCGCGCTGA AAAAGAAGAA 720
TTTGAAGAAA TTTCAGTTAG AGGTAGAGTG TTAGGAATCA GTTCACAAAC ACGATATCAA 780
CAACAAGAAA TTCCTATATA CCTTGATGAT TTAATTATCA TTTTAACGGA TGGTGTGACT 840

GAAGCTAGAA ATAGTGAAGG TACCTTTATA GATAAACAAA AACTTTTAGA ATATATTAAA 900
AAACATAAAC ATATGCACCC ACAAGATATT GTTCAAATTA TCTATGAAGC AATTTTAAAG 960
CTTCAAAACC CAAATAAAAA AGATGATATG ACTATTTTGA TTATAAAAAG AGTAAATTAA 1020

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Arg Arg Gln Leu Ile Val Glu Glu Phe Lys Gln His Tyr Lys Gly
1 5 10 15
Leu Ile Asp Glu Ser Leu Thr Cys Gln Asp Lys Val Glu Leu Ile Lys
20 25 30
Lys Cys Glu Lys Tyr Thr Asp Glu Val Ile Arg Lys Asp Val Leu Pro
35 40 45
Glu Asp Ile Val Asp Ile His Lys Asn Tyr Ile Leu Thr Leu Asn Leu
50 55 60
Thr Arg Glu Asp Val Phe Lys Thr Leu Asp Val Leu Gln Glu Ile Val
65 70 75 80
Lys Gly Phe Gly Tyr Ser Tyr Arg Asp Tyr Gln Arg Leu Val Asp Lys
85 90 95
Leu Gln Val His Asp Lys Glu Ile Asp Leu Ala Ser Ser Leu Gln Pro
100 105 110
Thr Met Leu Lys Thr Asp Ile Pro Gln Phe Asp Ser Ile Gln Ile Gly
115 120 125
Val Ile Ser Val Ala Ala Gln Lys Val Ser Gly Asp Tyr Phe Asn Leu
130 135 140
Ile Asp His Asn Asp Gly Thr Met Ser Phe Ala Val Ala Asp Val Ile
145 150 155 160
Gly Lys Gly Ile Pro Ala Ala Leu Ala Met Ser Met Ile Lys Phe Gly
165 170 175
Met Asp Ser Tyr Gly His Ser Gln Leu Pro Ser Asp Gly Leu Lys Arg
180 185 190

Leu Asn Arg Val Val Glu Lys Asn Ile Asn Gln Asn Met Phe Val Thr
195 200 205
Met Phe Tyr Gly Leu Tyr Glu Glu Met Asn His Leu Leu Tyr Cys Ser
210 215 220
Ser Ala Gly His Glu Pro Gly Tyr Ile Tyr Arg Ala Glu Lys Glu Glu
225 230 235 240
Phe Glu Glu Ile Ser Val Arg Gly Arg Val Leu Gly Ile Ser Ser Gln
245 250 255
Thr Arg Tyr Gln Gln Gln Glu Ile Pro Ile Tyr Leu Asp Asp Leu Ile
260 265 270
Ile Ile Leu Thr Asp Gly Val Thr Glu Ala Arg Asn Ser Glu Gly Thr
275 280 285
Phe Ile Asp Lys Gln Lys Leu Leu Glu Tyr Ile Lys Lys His Lys His
290 295 300
Met His Pro Gln Asp Ile Val Gln Ile Ile Tyr Glu Ala Ile Leu Lys
305 310 315 320
Leu Gln Asn Pro Asn Lys Lys Asp Asp Met Thr Ile Leu Ile Ile Lys
325 330 335
Arg Val Asn

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCAAAATTGC AAACGACCAA AATTGATTAA GTGCAATTAA ATAAATGTTA GTAAGTGAAT 60
CATAATTATC CTTGCTTAAG CATTTGCTTT GTAAGGGAAG TGAGGAGGCA ACTAATCGTG 120
GAAGAATTTA AGCAACATTA TAAGGGTTTA ATTGATGAAA GTTTAACGTG CCAAGATAAA 180
GTAGAATTGA TAAAAAAGTG TGAGAAATAC ACTGACGAAG TGATTCGTAA GGACGTCTTG 240
CCTGAAGACA TTGTCGATAT TCACAAAAAC TATATATTGA CGTTAAACTT AACGCGTGAA 300

GATGTGTTCA AGACATTAGA TGTCTTACAA GAAATCGTTA AAGGCTTTGG TTATAGTTAT 360
CGAGATTATC AAAGATTGGT AGATAAACTT CAAGTTCACG ATAAAGAGAT AGACTTAGCT 420
TCTAGCTTAC AACCAACAAT GCTTAAAACA GATATTCCAC AATTTGATAG TATTCAAATT 480
GGCGTTATTT CAGTGGCAGC ACAAAAAGTA AGTGGAGATT ATTTTAATTT AATTGACCAT 540
AACGATGGCA CAATGAGCTT TGCTGTTGCA GATGTCATTG GGAAAGGTAT ACCAGCTGCT 600
TTAGCAATGA GTATGATAAA GTTTGGCATG GATTCTTATG GACACTCACA ATTACCGAGT 660
GATGGCTTAA AACGTTTAAA TCGTGTTGTT GAAAAGAATA TTAATCAAAA TATGTTCGTC 720
ACAATGTTTT ATGGTTTATA TGAAGAAATG AACCATTTAT TGTATTGTAG TTCAGCTGGT 780
CATGAGCCTG GATATATTTA TCGCGCTGAA AAAGAAGAAT TTGAAGAAAT TTCAGTTAGA 840
GGTAGAGTGT TAGGAATCAG TTCACAAACA CGATATCAAC AACAAGAAAT TCCTATATAC 900
CTTGATGATT TAATTATCAT TTTAACGGAT GGTGTGACTG AAGCTAGAAA TAGTGAAGGT 960
ACCTTTATAG ATAAACAAAA ACTTTTAGAA TATATTAAAA AACATAAACA TATGCACCCA 1020
CAAGATATTG TTCAAATTAT CTATGAAGCA ATTTTAAAGC TTCAAAACCC AAATAAAAAA 1080
GATGATATGA CTATTTTGAT TATAAAAAGA GTAAATTAAT TTAAAAAAGA AGATTAGAAA 1140
TTATTTCGAT GGGTATATAA TAATTTGAAA TATAAATATG GTGGATACAG CGCTTAAAAT 1200
GAAGATAAAT ATTTTTAATA AGTAGGAGTG TAATGAAATG AATCTTAATA TAGAAACAAC 1260
CACTCAAGAT AAATTTTACG AAGTTAAAGT CGGTGGAGAA TTAGATGTTT ATACTGTGCC 1320
TGAATTAGAA GAGGTTTTAA CACCTATGAG ACAAGATGGA ACTCGTGATA TTTATGTTAA 1380
TTTAGAAAAT GTGAGTTATA TGGATTCGAC AGGTTTAGGT TTATTCGTAG GTACATTAAA 1440
AGCATTAAAC CAAAATGATA AAGAACTATA CATTTTAGGT GTGTCAGATC GTATCGGTAG 1500
ACTATTTGAA ATTACTGGTC TTAAGGATTT AATGCATGTT AATGAAGGAA CGGAGGTCGA 1560

ATAACATGCA ATCTAAAGAA GATTTTATCG AAATGCGCGT GCCAGCATCG GCAGAGTATG 1620
TAAGTTTAAT TCGTTTAACA CTTTCTGGCG TTTTTTCGAG AGCTGGTGCT ACATATGATG 1680
ATATTGAAGA TGCCAAGATT GCAGTTAGTG AAGCTGTGAC AAATGCAGTT AAACATGCAT 1740
ACAAAGAAAA TAACAATGTG GGCATTATTA ACATATATTT TGAAATTTTA GAAGATAAAA 1800
TTAAAATTGT TATTTCTGAT AAAGGTGACA GTTTTGATTA TGAAACAACT AAATCAAAAA 1860
TAGGTCCTTA CGATAAAGAC GAAAATATAG ACTTTTTACG CGAAGGTGGC CTAGGTTTAT 1920
TTTTAATCGA ATCTTTAATG GATGAAGTCA CAGTATATAA AGAATCTGGT GTGACAATCA 1980
GTATGACTAA GTATATAAAA AAAGAGCAGG TGCGAAATAA TGGCGAAAGA GTCGAAATCA 2040
GCTAATGAAG TTTCACCTGA GCAAATTAAC CAATGGATTA AAGAACACCA AGAAAATAAG 2100
AATACAGATG CACAGGATAA GTTAGTTAAA CATTACCAAA AACTAATTGA GTCATTGGCA 2160
TATAAATATT CTAAAGGACA ATCACATCAC GAAGATTTAG TTCAAGTTGG TATGGTTGGT 2220
TTAATAGGTG CCATAAATAG ATTCGATATG TCCTTTGAAC GGAAGTTTGA AGCCTTTTTA 2280
GTACCTACTG TAATCGGTGA AATCAAAAGA TATCTACGAG ATAAAACTTG GAGTGTACAT 2340
GTTCCGAGAC GTATTAAAGA AATTGGGCCA AGAATCAAAA AAGTGAGCGA TGAACTAACC 2400
GCTGAATTAG AGCGTTCACC TTCTATCAGT GAAATAGCTA ATCGATTAGA AGTCTCAGAA 2460
GAAGAAGTGT TAGAAGCAAT GGAAATGGGA CAAAGTTATA ATGCGTTAAG TGTTGATCAT 2520
TCCATTGAAG CTGATAAAGA TGGTTCAACT GTTACGCTAT TAGATATTAT GGGGCAACAA 2580
GATGACCATT ATGACTTAAC TGAAAAACGT ATGATTTTAG AAAAAATATT ACCTATATTA 2640
TCTGATCGCG AACGAGAAAT CATACAATGT ACGTTTATTG AAGGATTGAG TCAAAAAGAG 2700
ACAGGTGAGC GTATCGGTTT AAGTCAAATG CATGTATCAC GACTTCAGAG AACGGCAATT 2760
AAGAAATTAC AAGAAGCAGC ACATAAATAG AATTTGCTTA TTAATGATAC GTTTTATAAT 2820

GAAAAATCCA TATAATTATC CCTTGATTAT TAAATTGAAA TCGAGGGGTA TTTTTAATTT 2880
AATTAAGATT TTCGAATTAA TACATTATTA ACGTAGTTTA ATGTGTATCC ACATAAATGT 2940
CGCGATATAG TATTAATAAT TTAAGTGAAG AAGATATCTA ATTGTCGTTT TAAATAGGTG 3000
GATTGCTATT AGAATAAAAA AAGTAGTCTT AGATTATGAA ATTTAGAAAT GATGGTGTGT 3060
CATTTTCAAT AATCTTAGTG CGTTTTAAAA TATAGTATGA CCTAATAATT CGTTTTAAAT 3120
GTTTTGGGAA GTGAAAATTA CATTAAGTAT CATACCTTAA TAGAAGTATT TTAGAATATG 3180
TTAAAATAAA TGAGTAAATT TAAGAAAAAG TGTGGGTTAA GTAAATGGAC AATCAATTGA 3240
TTAATTCAAT CATAGAGAAA TATCAATTTA GTAAAAAACA AATTGAAGCA GTATTAACAC 3300
TGCTAGAAGA AAAAAATACA GTACCATTTA TTGCGAGGTA TCGAAAAGAG CAAACTGGTG 3360

Claims

1. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide having at least at 70% identity to a polynucleotide encoding a polypeptide comprising amino acids 1 to 333 of SEQ ID NO:2;
(b) a polynucleotide which is complementary to the polynucleotide of (a);
(c) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising amino acids 1 to 339 of SEQ ID NO:4; and
(d) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a), (b) or (c).

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 2 comprising a nucleotide selected from the group consisting of: nucleotide 1 to 1002 set forth in SEQ ID NO:1, nucleotides 1 to 1020 set forth in SEQ ID NO:3, and the polynucleotide set forth as SEQ ID NO:5.

5. The polynucleotide of Claim 2 comprising nucleotide selected from the group consisting of the nucleotide set forth in SEQ ID NO:1 encoding the polypeptide set forth in SEQ ID NO:2, the nucleotide set forth SEQ ID NO:3 encoding the polypeptide set forth SEQ ID NO:4, and and the polynucleotide set forth as SEQ ID NO:5.

6. The polynucleotide of Claim 2 which encodes a polypeptide comprising amino acids selected from the group consisting of: 1 to 333 of SEQ ID NO:2, and 1 to 339 of SEQ ID NO:4.

7. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide having at least a 70% identity to a polynucleotide encoding the same mature polypeptide expressed by the rsbU-1 gene contained in NCIMB Deposit No. 40771;
(b) a polynucleotide complementary to the polynucleotide of (a); and
(c) a polynucleotide comprising at least 15 bases of the polynucleotide of (a) or (b)

8. A vector comprising the DNA of Claim 2.

9. A host cell comprising the vector of Claim 8.

10. A process for producing a polypeptide comprising expressing from the host cell of Claim 9 a polypeptide encoded by said DNA.

11. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of Claim 8 such that the cell expresses the polypeptide encoded by the cDNA containing in the vector.

12. A polypeptide comprising an amino acid sequence which is at least 70% identical to amino acid selected from the group consisting of amino acid 1 to 333 of SEQ ID NO:2, and aniino acid 1 to 339 of SEQ ID NO:4.

13. A polypeptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence as set forth in SEQ ID NO:2, and the amino acid sequence as set forth in SEQ ID NO:4.

16. An antibody against the polypeptide of claim 12.

17. An antagonist which inhibits the activity of the polypeptide of claim 12.

18. A method for the treatment of an individual having need of rsbU-1 comprising: administering to the individual a therapeutically effective amount of the polypeptide of claim 12.

19. The method of Claim 17 wherein said therapeutically effective amount of the polypeptide is administered by providing to the individual DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

20. A method for the treatment of an individual having need to inhibit rsbU-1 polypeptide comprising: administering to the individual a therapeutically effective amount of the antagonist of Claim 16.

21. A process for diagnosing a disease related to expression of the polypeptide of claim 12 comprising:
determining a nucleic acid sequence encoding said polypeptide.

22. A diagnostic process comprising:
analyzing for the presence of the polypeptide of a claim 12 in a sample derived from a host

23. A method for identifying compounds which bind to and inhibit an activity of the polypeptide of claim 12 comprising:

contacting a cell expressing on the surface thereof a binding for the polypeptide, said binding being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said binding, with a compound to be screened under conditions to permit binding to the binding; and determining whether the compound binds to and activates or inhibits the binding by detecting the presence or absence of a signal generated from the interaction of the compound with the binding.

24. A method for inducing an immunological response in a mammal which comprises inoculating the mammal with rsbU-1, or a fragment or variant thereof, adaquate to produce antibody to protect said animal from upper respiratory tract (e.g. otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g. empyema, lung abscess), cardiac (e.g. infective endocarditis), gastrointestinal (e.g. secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g. cerebral abscess), eye (e.g. blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal & orbital cellulitis, darcryocystitis), kidney and urinary tract (e.g. epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g. impetigo, folliculitis, cutaneous abscess, celulitis, wound infection, bacterial myositis) bone and joint (e.g. septic arthritis, osteomyelitis).

25. A method of inducing immunological response in a mammal which comprises, through gene therapy, delivering gene encoding rsbU-1 fragment or a variant thereof, for expressing rsbU-1, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibody to protect said animal from disease

26. An immunological composition comprising a DNA which codes for and expresses a rsbU-1 polynucleotide or

protein coded therefrom which, when introduced into a mammal, induces an immunological response in the mammal to a given rsbU-1 polynucleotide or protein coded therefrom.

FIGURE 1. rsbU-1 cDNA sequence [SEQ ID NO:1]

(1002 nucleotides)

GTGGAAGAATTTAAGCAACATTATAAGGGTTTAATTGATGAAAGTTTAAGGTGCCAAGAT
AAAGTAGAATTGATAAAAAGTGTGAGAAATACACTGACGAAGTGATTCGTAAGGACGTC
TTGCCTGAAGACATTGTCGATATTCACAAAAACTATATATTGACGTTAAACTTAACGCGT
GAAGATGTGTTCAAGACATTAGATGTCTTACAAGAAATCGTTAAAGGCTTTGGTTATAGT
CATCGAGATTATCAAAGATTGGTAGATAAACTTCAAGTTCACGATAAAGAGATAGACTTA
GCTTCTAGCTTACAACCAACAATGCTTAAAACAGATATTCCACAATTTGATAGTATTCAA
ATTGGCGTTATTTCAGTGGCAGCACAAAAAGTAAGTGGAGATTATTTTAATTTAATTGAC
CATAACGATGGCACAATGAGCTTTGCTGTTGCAGATGTCATTGGGAAAGGTATACCAGCT
GCTTTAGCAATGAGTATGATAAAGTTTGGCATGGATTCTTATGGACACTCACAATTACCG
AGTGATGGCTTAAAACGTTTAAATCGTGTTGTTGAAAAGAATATTAATCAAAATATGTTC
GTCACAATGTTTTATGGTTTATATGAAGAAATGAACCATTTATTGTATTGTAGTTCAGCT
GGTCATGAGCCTGGATATATTTATCGCCCTGAAAAAGAAGAATTTGAAGAAATTTCAGTT
AGAGGTAGAGTGTTAGGAATCAGTTCACAAACACGATATCAACAACAAGAAATTCCTATA
TACCTTGATGATTTAATTATCATTTTAACGGATGGTGTGACTGAAGCTAGAAATAGTGAA
GCTACCTTTATAGATAAACAAAAACTTTTAGAATATATTAAAAACATAAACATATGCAC
CCACAAGATATTGTTCAAATTATCTATGAAGCAATTTTAAACCTTCAAAACCCAAATAAA
AAAGATGATATGACTATTTTGATTATAAAAAGAGTAAATTAA

FIGURE 2 **rsbU-1 predicted amino acid sequence [SEQ ID NO:2]**

(333 amino acids)

MEEFKQHYKGLIDESLTQQDKVELIKKCEKYTDEVIRKDVLPEDIVDIHKNYILTLNLTR

EDVEKTLDVLQEIVKGEGYSYRDYQRLVDKLQVHDKEIDLASSLQPTMLKTDIPQFDSIQ

IGVISVAAQKVSGDYFNLIDHNDGTMSFAVADVIGKGIPAALAMSMIKFGMDSYGHSQLP

SDGLKRLNRVVEKNINQNMFVTMFYGLYEEMNHLLYCSSAGHEPGYIYRAEKEEFEEISV

RGRVLGISSQTRYQQQEIPLYLDDLIIILTDGVTEARNSSGTEIDKQKLLEYIKKHKHMH

PQDIVQLIYEAILKLQNPKKQDMPLLIKRVE

FIGURE 3 rsbU-1cDNA sequence [SEQ ID NO:3]

(1020 nucleotides)

GTGAGGAGGCAACTAATCGTGGAAGAATTTAAGCAACATTATAAGGGTTTAATTGATGAA
AGTTTAACGTGCCAAGATAAAGTAGAATTGATAAAAAAGTGTGAGAAATACACTGACGAA
GTGATTCGTAAGGACGTCTTGCCTGAAGACATTGTCGATATTCACAAAAACTATATATTG
ACGTTAAACTTAACGCGTGAAGATGTGTTCAAGACATTAGATGTCTTACAAGAAATCGTT
AAAGGCTTTGGTTATAGTTATCGAGATTATCAAAGATTGGTAGATAAACTTCAAGTTCAC
GATAAAGAGATAGACTTAGCTTCTACCTTACAACCAACAATGCTTAAAACACATATTCCA
CAATTTGATAGTATTCAAATTGGCGTTATTTCAGTGGCAGCACAAAAAGTAAGTGGAGAT
TATTTTAATTTAATTGACCATAACGATGGCACAATGAGCTTTGCTGTTGCAGATGTCATT
GGGAAAGGTATACCAGCTGCTTTAGCAATGAGTATGATAAAGTTTGGCATGGATTCTTAT
GGACACTCACAATTACCGAGTCATGGCTTAAAACGTTTAAATCGTGTTGTTGAAAACAAT
ATTAATCAAAATATGTTCGTCACAATGTTTTATGGTTTATATGAAGAAATGAACCATTTA
TTGTATTGTAGTTCAGCTGGTCATGAGCCTGGATATATTTATCGCGCTGAAAAACAAGAA
TTTGAAGAAATTTCAGTTAGAGGTAGAGTGTTAGGAATCAGTTCACAAACACGATATCAA
CAACAAGAAATTCCTATATACCTTGATGATTTAATTATCATTTTAACGGATGGTGTGACT
GAAGCTAGAAATAGTGAAGGTACCTTTATAGATAAACAAAAACTTTTAGAACATATTAAA
AAACATAAACATATGCACCCACAAGATATTGTTCAAATTATCTATGAAGCAATTTTAAAG
CTTCAAAACCCAAATAAAAAAGATGATATGACTATTTTGATTATAAAAAGACTAAATTAA

FIGURE 4 rsbU-1 predicted amino acid sequence [SEQ ID NO:4]

(339 amino acids)

MRRQLIVEEFKQHYKGLIDESLTCQDKVELIKKCEKYTDEVIRKDVLPEDIVDIHKNYIL
TLNLTREDVFKTLDVLQEIVKGFGYSYRDYQRLVDKLQVHDKEIDLASSLQPTMLKTDIP
QFDSIQIGVISVAAQKVSGDYFNLIDHNDGTMSFAVADVIGKGIPAALAMSMIKFGMDSY
GHSQLPSDGLKRLNRVVEKNINQNMFVTMFYGLYEEMNHLLYCSSAGHEPGYIYRAEKEE
FEEISVRGRVLGISSQTRYQQQEIPIYLDDLIILLTDGVTEARNSEGTEIDKQKLLEYIK
KHKHMHPQDIVQLIYEAILKLQNPNKKDDMTILIIKRVN

FIGURE 5 **Polynucleotide sequence of the sigB putative operon [SEQ ID NO:5]**

(3360 nucleotides)

GCAAAATTGCAAACGACCAAAATTGATTAAGTGCAATTAAATAAATGTTAGTAAGTGAAT
CATAATTATCCTTGCTTAAGCATTTGCTTTGTAAGGGAAGTGAGGAGGCAACTAATCGTG
GAAGAATTTAAGCAACATTATAAGGGTTTAATTGATGAAAGTTTAACGTGCCAAGATAAA
GTAGAATTGATAAAAAAGTGTGAGAAATACACTGACGAAGTGATTCGTAAGGACGTCTTG
CCTGAAGACATTGTCGATATTCACAAAAACTATATATTGACGTTAAACTTAACGCGTGAA
GATGTGTTCAAGACATTAGATGTCTTACAAGAAATCGTTAAAGGCTTTGGTTATAGTTAT
CGAGATTATCAAAGATTGGTAGATAAACTTCAAGTTCACGATAAAGAGATAGACTTAGCT
TCTAGCTTACAACCAACAATGCTTAAAACAGATATTCCACAATTTGATAGTATTCAAATT
GGCGTTATTTCAGTGGCAGCACAAAAAGTAAGTGGAGATTATTTTAATTTAATTGACCAT
AACGATGGCACAATGAGCTTTGCTGTTGCAGATGTCATTGGGAAAGGTATACCAGCTGCT
TTAGCAATGAGTATGATAAAGTTTGGCATGGATTCTTATGGACACTCACAATTACCGAGT
GATGGCTTAAAACGTTTAAATCGTGTTGTTGAAAAGAATATTAATCAAAATATGTTCGTC
ACAATGTTTTATGGTTTATATGAAGAAATGAACCATTTATTGTATTGTAGTTCAGCTGGT
CATGAGCCTGGATATATTTATCGCGCTGAAAAAGAAGAATTTGAAGAAATTTCAGTTAGA
GGTAGAGTGTTAGGAATCAGTTCACAAACACGATATCAACAACAAGAAATTCCTATATAC
CTTGATGATTTAATTATCATTTTAACGGATGGTGTGACTGAAGCTAGAAATAGTGAAGGT
ACCTTTATAGATAAACAAAAACTTTTAGAATATATTAAAAAACATAAACATATGCACCCA
CAAGATATTGTTCAAATTATCTATGAAGCAATTTTAAAGCTTCAAAACCCAAATAAAAAA
GATGATATGACTATTTTGATTATAAAAAGAGTAAATTAATTTAAAAAAGAAGATTAGAAA

TTATTTCGATGGGTATATAATAATTTGAAATATAAATATGGTGGATACAGCGCTTAAAAT
GAAGATAAATATTTTTAATAAGTAGGAGTGTAATGAAATGAATCTTAATATAGAAACAAC
CACTCAAGATAAATTTTACGAAGTTAAAGTCGGTGGAGAATTAGATGTTTATACTGTGCC
TGAATTAGAAGAGGTTTTAACACCTATGAGACAAGATGGAACTCGTGATATTTATGTTAA
TTTAGAAAATGTGAGTTATATGGATTCGACAGGTTTAGGTTTATTCGTAGGTACATTAAA
AGCATTAAACCAAAATGATAAAGAACTATACATTTTAGGTGTGTCAGATCGTATCGGTAG
ACTATTTGAAATTACTGGTCTTAAGGATTTAATGCATGTTAATGAAGGAACGGAGGTCGA
ATAACATGCAATCTAAAGAAGATTTTATCGAAATGCGCGTGCCAGCATCGGCAGAGTATG
TAAGTTTAATTCGTTTAACACTTTCTGGCGTTTTTTCGAGAGCTGGTGCTACATATGATG
ATATTGAAGATGCCAAGATTGCAGTTAGTGAAGCTGTGACAAATGCAGTTAAACATGCAT
ACAAAGAAAATAACAATGTGGGCATTATTAACATATATTTTGAAATTTTAGAAGATAAAA
TTAAAATTGTTATTTCTGATAAAGGTGACAGTTTTGATTATGAAACAACTAAATCAAAAA
TAGGTCCTTACGATAAAGACGAAAATATAGACTTTTTACGCGAAGGTGGCCTAGGTTTAT
TTTTAATCGAATCTTTAATGGATGAAGTCACAGTATATAAAGAATCTGGTGTGACAATCA
GTATGACTAAGTATATAAAAAAAGAGCAGGTGCGAAATAATGGCGAAAGAGTCGAAATCA
GCTAATGAAGTTTCACCTGAGCAAATTAACCAATGGATTAAAGAACACCAAGAAAATAAG
AATACAGATGCACAGGATAAGTTAGTTAAACATTACCAAAAACTAATTGAGTCATTGGCA
TATAAATATTCTAAAGGACAATCACATCACGAAGATTTAGTTCAAGTTGGTATGGTTGGT
TTAATAGGTGCCATAAATAGATTCGATATGTCCTTTGAACGGAAGTTTGAAGCCTTTTTA
GTACCTACTCTAATCGGTGAAATCAAAAGATATCTACGAGATAAAACTTGGAGTGTACAT
GTTCCGAGACGTATTAAAGAAATTGGGCCAAGAATCAAAAAAGTGAGCGATGAACTAACC
GCTGAATTAGAGCGTTCACCTTCTATCAGTGAAATAGCTAATCGATTAGAAGTCTCAGAA

GAAGAAGTGTTAGAAGCAATGGAAATGGGACAAAGTTATAATGCGTTAAGTGTTGATCAT

TCCATTGAAGCTGATAAAGATGGTTCAACTGTTACGCTATTAGATATTATGGGGCAACAA

GATGACCATTATGACTTAACTGAAAAACGTATGATTTTAGAAAAAATATTACCTATATTA

TCTGATCGCGAACGAGAAATCATACAATGTACGTTTATTGAAGGATTGAGTCAAAAAGAG

ACAGGTGAGCGTATCGGTTTAAGTCAAATGCATGTATCACGACTTCAGAGAACGGCAATT

AAGAAATTACAAGAAGCAGCACATAAATAGAATTTGCTTATTAATGATACGTTTTATAAT

GAAAAATCCATATAATTATCCCTTGATTATTAAATTGAAATCGAGGGGTATTTTTAATTT

AATTAAGATTTTCGAATTAATACATTATTAACGTAGTTTAATGTGTATCCACATAAATGT

CGCGATATAGTATTAATAATTTAAGTGAAGAAGATATCTAATTGTCGTTTTAAATAGGTG

GATTGCTATTAGAATAAAAAAAGTAGTCTTAGATTATGAAATTTAGAAATGATGGTGTGT

CATTTTCAATAATCTTAGTGCGTTTTAAAATATACTATCACCTAATAATTCGTTTTAAAT

GTTTTGGGAAGTGAAAATTACATTAAGTATCATACCTTAATAGAAGTATTTTAGAATATG

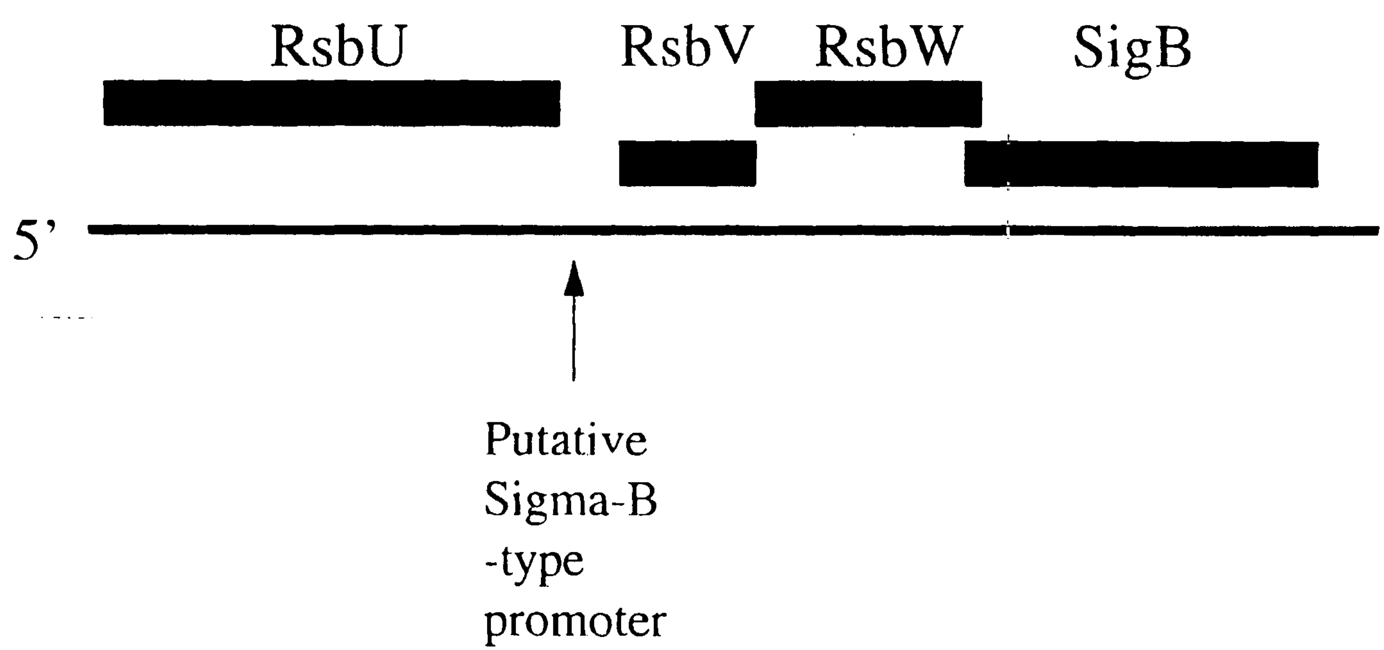
TTAAAATAAATGAGTAAATTTAAGAAAAAGTGTGGGTTAAGTAAATGGACAATCAATTGA

TTAATTCAATCATAGAGAAATATCAATTTAGTAAAAAACAAATTGAAGCAGTATTAACAC

TGCTAGAAGAAAAAAATACAGTACCATTTATTGCGAGGTATCGAAAAGAGCAAACTGGTG

FIGURE 6

Arrangements of genes in *S. aureus* genome



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(54) **RsbU-1 derived from Staphylococcus**

(57) isbU-I polypeptides and DNA (RNA) encoding such rsbU-1 and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such rsbU-1 forlm the treament of infection, particularly bacterial infections. Antagonists against such rsbU-1 and their use as a therapeutic to treat infections, particularly bacterial infections are also disclosed. Also disclosed are diagnostic assay for detecting diseases related to the presence of rsbU-1 nucleic acid sequences and the polypeptides in a host. Also disclosed are diagnostic assays for detecting polynucleotides encoding SigB operon and for detecting the polypeptide in a host.

EP 0 843 011 A3

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number
EP 97 30 8512

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	WU S ET AL: "Sigma-B, a putative operon encoding alternate sigma factor of Staphylococcus aureus RNA polymerase: molecular cloning and DNA sequencing." JOURNAL OF BACTERIOLOGY, (1996 OCT) 178 (20) 6036-42. JOURNAL CODE: HH3. ISSN: 0021-9193., XP002103953 United States * figures 2-4 * ---	1-3,5-13	C12N15/31 C07K14/31 C07K16/12 C12Q1/68 G01N33/50 C07H21/00 A61K31/70 A61K48/00
P,X	EP 0 786 519 A (HUMAN GENOME SCIENCES INC) 30 July 1997 * SEQ ID Nos 714,836,882,1108,2158,2581 * ---	7	
P,X	WO 97 30070 A (HODGSON JOHN EDWARD ;NICHOLAS RICHARD OAKLEY (GB); PRATT JULIE M () 21 August 1997 * SEQ ID Nos 152,767 * * page 80 * -----	1-3,5,7-12	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C12N
C07K
C12Q
G01N
C07H
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely

Claims searched incompletely

Claims not searched .

Reason for the limitation of the search

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	27 May 1999	ESPEN, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 97 30 8512

Although claims 18,19,24,25 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) not searched:
17,20

Reason for the limitation of the search:

Said claims relate to antagonists without giving a true technical characterization of the claimed matter. In consequence, the scope of said claims is ambiguous and, moreover, their subject-matter is vague and not sufficiently disclosed.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 97 30 8512

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report
The members are as contained in the European Patent Office EDP file on
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